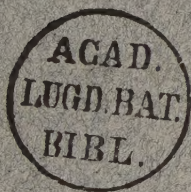


AN EXPERIMENTAL STUDY OF THE RHYTHMIC
ACTIVITY OF ISOLATED STRIPS OF
THE HEART-MUSCLE.

DISSERTATION

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE
JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

BY E. G. MARTIN.



BALTIMORE, MD.

MAY, 1904.

AN EXPERIMENTAL STUDY OF THE RHYTHMIC
ACTIVITY OF ISOLATED STRIPS OF
THE HEART-MUSCLE.

By E. G. MARTIN.

[From the Physiological Laboratory of the Johns Hopkins University, Baltimore, Md.]

THIS study was undertaken at the suggestion and under the direction of Dr. Howell, with the hope of throwing some additional light upon the problem of the interaction of ions, especially of sodium and calcium, in rhythmically contractile tissues.

Strips from the apex of the ventricle were used, prepared as described by Greene.¹ In many experiments four strips from the same heart were used. These were obtained by cutting the usual apex strips crosswise in the middle. The records given by these strips, though small, were in every other respect comparable with those obtained from pieces of tissue of the usual size. The great advantage of this procedure lay in the fact that three controlled experiments could be carried on at once, instead of one, as by the usual method, hence a greater range of comparison was possible. The distilled water and the salts used in the experiments were prepared as described by Greene.² The salts used were the chlorides of sodium, calcium, and potassium.

Lingle³ has stated that the action of ions in initiating spontaneous contractions may be looked upon as entirely distinct from the action of these same ions in maintaining them after they have begun. It has seemed to the author reasonable to suppose that if, as has been suggested by several observers, spontaneous contractions are the sign of a certain relationship existing among the ions in the tissue, then whenever the proper relationship is present, whether naturally or as the result of artificial manipulation, spontaneous contractions will occur, whether or not the tissue has been previously active. In other

¹ GREENE: This journal, 1898, ii, p. 83.

² GREENE: *Loc. cit.*, p. 86.

³ LINGLE: This journal, 1900, iv, p. 270.

words, whatever condition suffices to initiate beats should, if maintained, be sufficient to cause their continuance. In order to test this idea, the experiments described in this connection were carried on. The object immediately in view was the study of every possible method of initiating beats in resting strips, and of every environment under which they continue active after beats have commenced. Such a study is, of necessity, a work requiring very much time for its completion, but some of the results thus far obtained may throw enough light on the problem under consideration to make their presentation at this time worth while.

The experiments here recorded were performed during the year 1903. During the winter and autumn months the work was carried on in this laboratory, and the animal studied was the common slider terrapin, *Pseudemys hieroglyphica*. During the spring and summer the author worked in a temporary laboratory established by himself in Minnesota. The work in this summer laboratory was almost entirely upon the common mud turtle, *Chrysemys marginata*. A few specimens of *Chelydra serpentina* were also studied. It should be stated at the outset that specific differences in the reactions of heart-tissue, if any were present, were neither conspicuous nor constant enough to necessitate differentiation in the study of the experiments. Seasonal variations seemed quite marked in some instances. These will be considered in their proper place. What seems to the author to be the typical reaction is obtained from the heart of an animal in winter condition, studied shortly after it is removed from its natural habitat.

THE INITIAL STANDSTILL.

Whenever a strip is cut from a beating ventricle, it immediately comes to rest in diastole. Gaskell¹ states that this standstill is frequently only temporary, and that, after a longer or shorter interval, the strip resumes rhythmic activity spontaneously. Observers in recent years have not been able to obtain this result, except at rare intervals, on strips suspended in air, and left entirely without treatment. We may, therefore, safely conclude that, under normal conditions, the isolated ventricle is not ordinarily spontaneously rhythmical. Lingle² attributes the standstill of the isolated ventricular strip to the mechanical shock of the operation by which it

¹ GASKELL: Philosophical transactions, London, 1882, p. 993.

² LINGLE: This journal, 1903, viii, p. 76.

is prepared for study. It would seem, however, that if mechanical shock is the only factor concerned in the failure of the strip to beat, spontaneous recovery should be the rule, rather than the very rare exception. An experiment will be cited presently in which a strip, under proper conditions, was isolated completely without disturbance of the continuity of its rhythm, although suffering as much mechanical violence as any strip does in preparation.

Howell¹ has suggested the view that the standstill of the isolated ventricle is due to the presence in the circulating medium of some substance whose influence upon the ventricle is inhibitory. According to his view, the potassium ions play this inhibitory rôle. As is well known, the simplest and, in fact, the usual method of inducing beats in a strip of ventricle, is by immersing the strip in 0.7 per cent sodium chloride solution, in which, if alive, it will invariably begin, sooner or later, to beat rhythmically. Precisely the same result will follow if the solution contain, in addition to sodium chloride, the physiological proportion of calcium chloride, — about 0.025 per cent. If, however, there be present in the solution, in addition to these, the physiological proportion of potassium chloride, about 0.03 per cent, the strip will, in most cases, remain quiet indefinitely. Permanent quiescence can be maintained, in virtually every case, by increasing slightly the proportion of potassium chloride in the solution. Granting that rhythmic activity depends upon ionic interaction, the facts cited above certainly indicate the correctness of Howell's view. The tissue contains sodium, calcium, and potassium ions at the outset; the only one of these whose outward diffusion must be permitted, in order for the strip to become active, is the potassium. The question as to which are the efficient ions in *producing* activity and *maintaining* it, is entirely distinct from the one now under consideration, and will be discussed in its proper place.

Reference was made above to an experiment in which the mechanical violence of preparing the strip did not cause standstill. The experiment was as follows: A heart was removed from the body and the apex of the ventricle partially severed by two cuts parallel with the margin, as shown in Fig. 1. The preparation was then mounted, as shown in the figure, with the partially severed apex in connection with a recording lever. The apex beat throughout the entire process of preparation synchronously with the remainder of the heart, and continued to do so after suspension. One such preparation was sus-

¹ HOWELL: This journal, 1898, ii, p. 79.

pendent in moist air for twenty minutes, during which time the beat of the apex was continuous and vigorous. At the end of this time, the apex was isolated by a cut through the connecting bridge of tissue, whereupon it came promptly to rest, and remained perfectly still throughout the experiment, although the great mass of the organ continued to beat as before. A precisely

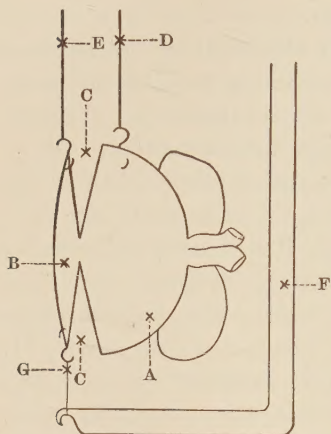


FIGURE I. — Diagram illustrating the method of suspending a whole heart with partially isolated apex strip. *A*, entire terrapin heart; *B*, apex almost isolated by cuts *C* and *C'*; *D*, thread suspending whole heart to fixed support; *E*, thread connecting apex with recording lever; *F*, glass rod to which apex is attached at lower end by a thread *G*. The whole arranged to be immersed at pleasure in any desired solution.

similar preparation was suspended for twenty minutes in 0.7 per cent sodium chloride solution, after which the apex was isolated in the same way as in the former instance. In this case, however, the isolated apex did not come to rest, but continued to beat regularly, with no pause save a momentary one while the cut was being made. The same amount of mechanical violence was inflicted in each case. The conclusion to be drawn from this experiment is that the standstill of the isolated ventricle is due, not to mechanical violence, but to some factor whose influence is overcome by the treatment with the saline solution. An interesting fact was brought out by this experiment, although bearing upon quite a different problem from the one under consideration. The rhythm of the entire heart, including the partially separated apex, was, in one experiment, twenty-eight per minute. The rhythm of the apex fell, immediately upon isolation, to seven per minute, which rate was maintained for some time. During the later stages of the experiment the rate gradually in-

creased, although the original value was never regained. This experiment indicates that a ventricle in which spontaneous contractions have been artificially induced has a rhythm of its own, which is quite independent of the normal rhythm of the heart as a whole.

THE LATENT PERIOD.

In a preceding paragraph the idea was advanced that the isolated ventricular strip remains at a standstill for the reason that it is not, under the conditions of normal life, spontaneously rhythmic. It is the purpose of this section to describe the various artificial conditions under which rhythmic contractions may occur.

Sodium chloride solution. — The first method of inducing beats has already been mentioned. It consists of immersing the strip in 0.7 per cent sodium chloride solution. A strip in this solution exhibits a period of standstill before the onset of activity. This latent period is of variable length; its average duration, for eighty-five observations made by the author throughout the year, was forty minutes. There appears to be a distinct seasonal variation in this respect. The average latent period for fifty-one observations taken during the months of November, December, January, and February was fifty minutes, while for thirty-one observations during the months of May, June, August, and September the average latent period was twenty-seven minutes. This variation is probably associated with the difference of temperature of the two seasons, although observations which will be reported presently indicate that there is also a definite difference in the condition of the tissue, to which this difference in the latent period may be referred. The extreme range of duration of the latent period was between five and one hundred and thirty-six minutes. Instances of such wide departure from the average are decidedly in the minority, seventy-five per cent of all observations falling reasonably near the average value.

Preliminary bath containing calcium. — If a fresh strip be placed for a short time (three to five minutes) in a solution containing 0.7 per cent sodium chloride and 0.025 per cent calcium chloride, and be then transferred to a solution of 0.7 per cent sodium chloride alone, the duration of the latent period is very much less than that shown by strips placed directly in 0.7 per cent sodium chloride without the preliminary treatment with the calcium-containing solution. This result was constant during the winter months, and is so marked that it cannot be regarded as accidental. Table I gives the results of fourteen observations of this fact. The averages from the table are as follows: for those strips which were treated with the solution containing calcium chloride, the average latent period was eight minutes, while for the control strips, which did not receive this

treatment, but were placed directly in 0.7 per cent sodium chloride, the average latent period was thirty-nine minutes. That this effect is due to the calcium of the preliminary solution, is made clear by

TABLE I.

Effect of a brief preliminary bath of a solution containing 0.025 per cent calcium chloride, and 0.7 per cent sodium chloride on the latent period of fresh strips in 0.7 per cent sodium chloride.

Date of observation. 1903.	Latent period of control in 0.7 % NaCl.	Latent period of test strip.	Date of observation. 1903.	Latent period of control in 0.7 % NaCl.	Latent period of test strip.
	minutes	minutes		minutes	minutes
Jan. 23	44	9.5	Feb. 18	..	6.5
" 27	..	5.0	" 18	..	7.0
" 27	..	7.0	" 27	112	13.0
" 28	24	4.0	" 28	35	7.0
" 28	24	11.0	Mar. 2	24	13.0
" 29	10	5.0	" 2	21	10.0
Feb. 5	42	10.0	" 3	51	10.0

TABLE II.

Effect of a brief preliminary bath of 0.025 per cent calcium chloride in sugar solution on the latent period of fresh strips in 0.7 per cent sodium chloride.

Date of observation. 1903.	Latent period of control in 0.7 % NaCl.	Latent period of test strip.
	minutes	minutes
Jan. 24	..	6.0
" 26	56	5.0
" 26	..	9.5
Dec. 15	50	15.0
" 17	70	10.0

the observations recorded in Table II, in which exactly the same proportion of calcium was used as in the former series of observations, but the solution was made isotonic with the serum by means of cane sugar instead of sodium chloride. The average latent period of the

calcium-treated strips in this series of five observations was nine minutes, the control strips had an average latent period of fifty-nine minutes. Greene¹ observed a similar shortening of the latent period when a strong solution of calcium chloride in distilled water was used as a preliminary bath, followed by 0.7 per cent sodium chloride solution. Table III shows that the presence of potassium in small

TABLE III.

Effect of a brief preliminary bath of normal Ringer's solution on the latent period of fresh strips in 0.7 per cent NaCl.

Date of observation. 1903.	Latent period of control in 0.7 % NaCl.	Latent period of test strip.
	minutes	minutes
Jan. 7	* ..	5.0
" 14	..	5.0
" 15	..	3.0
" 20	..	5.5
" 21	22.5	7.5
" 22	60.0	8.0
Feb. 4	..	3.0
Dec. 21	60.0	10.0

amounts in the preliminary solution does not interfere with the action of the calcium. The modification of Ringer's solution described by Howell² was used as the preliminary bath in this series of experiments. The average latent period of the calcium-treated strips in this series of eight observations was six minutes. The earlier observations of this series were not controlled; the average latent period of the three controls which were made later, was forty-seven minutes. Several experiments were performed in which small proportions of potassium chloride, from 0.03 per cent to 0.04 per cent were used with 0.7 per cent sodium chloride in the preliminary bath. The presence of the potassium compound did not tend to produce any shortening of the latent period, so far as could be determined from the few tests that were made. If all the observations of the effect of physiological proportions of calcium chloride in the

¹ GREENE: *Loc. cit.* p. 101.

² HOWELL: This journal, 1898, ii, p. 53.

preliminary bath be grouped together, the following values are obtained: twenty-seven calcium-treated strips showed an average latent period of 7.5 minutes. Their controls, which had precisely similar treatment, except that they had not the preliminary immersion in a calcium-containing solution, showed an average latent period of forty-four minutes. These observations seem to establish the fact that calcium, under the conditions quoted, has the power to induce contractions in freshly isolated ventricular strips, immersed in 0.7 per cent sodium chloride solution, much more promptly than sodium chloride used alone.

A number of observations, twenty-seven in all, were made in regard to the effect of larger proportions of calcium chloride in the preliminary bath. Solutions were used in which the proportion of calcium chloride ranged from twice to four times the physiological, *i. e.*, from 0.05 per cent to 0.1 per cent. In these experiments the calcium chloride, in eleven cases, caused distinct shortening of the latent period, while in sixteen experiments this was either distinctly lengthened, or was only slightly influenced. The results of this series of experiments are given in Table IV. A careful study of this table brings out a striking fact, which can be made clear by a statement of the average values of the different sets of latent periods. For the eleven cases in which the large dose of calcium shortened the latent period, its average duration was fifteen minutes; the average latent period for the eleven controls was thirty-eight minutes. For the sixteen cases in which calcium delayed the onset of activity, the average latent period was thirty-six minutes; the sixteen controls for these, however, showed an average latent period of only twenty-two minutes. With the exception of three observations, all those in which the large proportion of calcium chloride delayed the onset of activity were made during the summer months, when, as was noted above, the latent period of fresh strips in 0.7 per cent sodium chloride was considerably shorter than in cold weather. The point upon which it is particularly desired to lay stress is this. *The shortening of the latent period in sodium chloride observed during warm weather depends upon the same condition of the tissue as does the delayed activity which calcium sometimes causes.* A study of the average latent periods of the author's summer experiments makes this fact apparent. Of the thirty-one observations of the latent period made during warm weather, thirteen showed an inhibitory action on the part of calcium, eleven showed the opposite effect, while the point

TABLE IV.

Effect of large doses of calcium chloride in the preliminary bath, on the latent period of fresh strips in 0.7 per cent NaCl.

Date of observation. 1903.	Composition of solution.	Latent period of control in 0.7% NaCl.	Latent period of test strip.
Aug. 29	0.05 % CaCl_2 and 0.7 % NaCl.	minutes 65	minutes 17
Sept. 3	" " " " "	25	6
Dec. 17	" " " " "	45	10
" 19	" " " " "	25	8
May 6	0.06 " " " "	62	29
" 21	" " " " "	22	7
June 4	" " " " "	21	14
May 7	0.01 % " " " "	30	18
" 8	" " " " "	58	26
Aug. 20	" " " " "	36	17
" 27	" " " " "	36	16
May 15	0.04 % " " " "	5	10
Aug. 5	" " " " "	10	14
" 11	" " " " "	23	22
" 19	0.05 % " " " "	10	24
" 31	" " " " "	40	35
Sept. 5	" " " " "	28	37
" 7	" " " " "	13	16
" 11	" " " " "	5	10
" 12	" " " " "	11	27
Nov. 28	" " " " "	70	92
Dec. 4	" " " " "	35	50
" 10	" " " " "	45	75
May 23	0.06 % " " " "	13	70
" 26	" " " " "	35	80
Aug. 10	0.01 % " " " "	8	10
Sept. 2	" " " " "	4	11

was not determined for the remaining seven. The average latent period for the control strips in sodium chloride, of the thirteen whose test strips showed calcium inhibition, was sixteen minutes, while for the remaining eighteen, the average latent period was thirty-five minutes. The possible significance of these facts will be discussed in a later paragraph.

The facts presented above may be summarized in the following statement of the effect of a preliminary bath of calcium chloride on fresh ventricular strips. This substance in physiological proportion applied to a strip for a short time diminishes markedly the latent period of the strip in 0.7 per cent sodium chloride. In twice the physiological proportion or more, calcium chloride may have the same effect as in physiological concentration upon strips whose condition is typical, but upon strips in an atypical condition, as indicated by a marked shortening of the normal latent period in 0.7 per cent sodium chloride, such concentrations of calcium chloride are likely to exert a distinctly inhibitory influence.

Continued application of calcium-containing solutions.—The effects of calcium which have been thus far described were all obtained by treating strips for a short time with a solution containing calcium ions, and then transferring them to a solution of 0.7 per cent sodium chloride in which there were no calcium ions except those that might have been carried over on the tissue. That the effects observed were due to the brief contact with the calcium-containing solution, rather than to the calcium ions which were carried over when the strip was transferred to the pure sodium chloride solution, was shown by the fact that thorough rinsing with several changes of saline solution, or, for that matter, immersing the strip in a flowing stream of 0.7 per cent sodium chloride solution, did not change the effect of the previous application of calcium chloride.

A few experiments, ten in all, were performed in order to see whether the calcium effect would be the same when the strip was immersed in a solution containing both sodium and calcium chlorides, and left there till beats began. Concentrations of calcium chloride ranging from the physiological, 0.025 per cent, to three times that amount, were used. The results were very conflicting. In four experiments the presence of calcium was without effect on the latent period; in four the latent period was distinctly lengthened; and in the two remaining experiments the latent period was markedly shortened. Two of the experiments in which calcium had no effect on the latent

period were performed in the month of May; all the others of this series were carried on in November and December. It seems scarcely reasonable to suppose that the effect of a brief preliminary bath of a calcium-containing solution should be markedly different from that of the same solution applied for a longer time; therefore a more exhaustive study of this latter point is desirable.

Mixed solutions of sodium chloride and cane sugar.—Lingle¹ has shown that heart strips may beat spontaneously in mixtures of 0.7 per cent sodium chloride and isotonic cane sugar solution in which the dilution of the sodium chloride is very considerable. The author studied the latent period of strips in such solutions. In the paper just referred to, Lingle² notes the fact that the latent period in such dilute solutions is longer than in physiological salt solution.

Two dilutions of sodium chloride with sugar solution were studied by the author. In one the proportion was 10 c.c. 0.7 per cent sodium chloride to 15 c.c. isotonic sugar solution; in the other the proportion was 5 c.c. 0.7 per cent sodium chloride to 20 c.c. of the sugar solution. There were no differences between the latent periods of these two sets of observations that might not fall within the range of individual variation; therefore the entire group is studied together. The average latent period of the control strips in 0.7 per cent sodium chloride, for the entire series of fourteen observations, was fifty-seven minutes. The average latent period of the strips bathed in solutions of sodium chloride diluted with sugar was one hundred and seventy minutes. It should be noted in passing that occasionally a strip of ventricle is observed which remains quiet indefinitely in a solution in which the proportion of sodium chloride is much below the physiological. This occurs more frequently, as Lingle points out, the more the sodium chloride is diluted.

Long separation from the body.—A number of observers have called attention to the fact that perfectly fresh heart-tissue is more resistant to interchanges among its salts than the same tissue is after it has been separated from its normal connections for some time. This observation is corroborated by the results of two series of experiments performed by the author. The first series consisted in preparing strips according to the usual method, and suspending them directly in moist air. After hanging thus for some time, each strip was immersed in 0.7 per cent sodium chloride and the latent period

¹ LINGLE: This journal, 1902, viii, p. 91.

² LINGLE: *Loc. cit.* p. 91.

in the solution under this condition was compared with that of a perfectly fresh strip in the same solution. The average values for twelve experiments, in which the time of suspension in the moist chamber ranged from one-half to four and one-half hours, are as follows: the latent period of the control strips, placed directly upon isolation into 0.7 per cent sodium chloride, averaged forty-eight minutes; the latent period of the test strips, transferred to 0.7 per cent sodium chloride after long suspension in moist air, averaged fourteen minutes. In the second series the fresh strips were placed in solutions containing 0.7 per cent sodium chloride together with 0.04 per cent potassium chloride. This proportion of potassium chloride is one-fourth larger than the physiological, reckoning the latter to be that used in Ringer's solution, and usually suffices to prevent spontaneous contractions indefinitely. After immersion in this solution from one to three hours, the strips were transferred to 0.7 per cent sodium chloride, and the latent period after transfer compared with that of perfectly fresh strips as before. The results for this series of four experiments are these: the latent period of the strips transferred from the potassium-containing solution to that of sodium chloride alone, averaged in the latter solution twelve minutes. The controls showed an average latent period of fifty-five minutes. In every case the test strip was left in the potassium-containing solution for some time after its companion strip in pure sodium chloride had commenced to beat. In no case was there any sign of activity in the strip, so long as it remained in the solution containing potassium chloride, although it began to beat soon after transfer to a solution of sodium chloride without potassium ions in it. These facts certainly indicate that potassium exerts an inhibitory influence upon the tissue, which is removed by allowing diffusion of potassium to take place. If, as Howell has suggested, the only factor concerned in the normal latent period of fresh strips in sodium chloride is the presence of excess of potassium in the tissue, these experiments also show that diffusion is less rapid in fresh strips than in those isolated for some time.

Solution of sugar isotonic with the blood.—Lingle¹ states that strips which have been suspended in a solution of cane sugar, isotonic with the blood, for some time, frequently begin to beat very promptly upon removal to a solution of 0.7 per cent sodium chloride. The author has been unable to obtain this result from strips which were placed in the sugar solution immediately upon isolation, and kept

¹ LINGLE: This journal, 1900, iv, p. 270.

there one hour or more. Such strips have invariably gone into a condition of strong tone, and the first effect of removal to a salt solution has always been a prompt and marked fall of tone, followed ultimately, but only after a number of minutes, in one case one hour, by spontaneous contractions. It should be stated, however, that if the sugar solution contained a small proportion of sodium chloride, not enough to bring about activity itself, transfer to 0.7 per cent sodium chloride was usually followed by contractions very promptly indeed; unless the strip had been too long in the sugar solution, in which event no contractions resulted from the application of the sodium chloride.

Organic calcium-containing solutions, including blood.— In connection with the study of the influence of calcium in shortening the

TABLE V.

Effect of a brief preliminary bath of terrapin serum upon the latent period of a fresh strip of terrapin ventricle in 0.7 per cent NaCl.

Date of observation. 1903.	Latent period of control in 0.7 % NaCl.	Latent period of test strip.
	minutes	minutes
Feb. 3	70	15.0
" 4	..	4.5
" 20	27	12.0
" 28	35	15.0
Sept. 3	26	5.0

latent period of fresh strips in 0.7 per cent sodium chloride solution, a point of considerable interest was observed which should be recorded in this connection. After the effect of calcium in inorganic combination, as represented by calcium chloride, had been satisfactorily determined, the question arose as to whether calcium in organic combination would have the same effect. Milk and solutions of calcium glycono-phosphate in sodium chloride solution were tried and found to produce a definite shortening of the latent period. But the serum of the turtle contains calcium in organic or inorganic combination, and ought, therefore, to behave in this respect like other calcium-containing solutions. Serum was obtained by defibrinating the blood of a terrapin, and allowing the corpuscles to settle. A fresh strip was given a preliminary bath for three minutes in twelve cubic

centimetres of this serum. The results of these experiments are given in Table V. The average latent period for the control strips was forty minutes; for the test strips, ten minutes. In a single instance the fresh blood was left at a low temperature for twenty-four hours, and the clear, unclotted plasma thus obtained was tested. During the three minutes that this plasma was about the strip, clotting began in it. It reduced the latent period of the test strip to thirteen minutes, while the control strip had a latent period of thirty-four minutes. Fresh blood itself was drawn, and used immediately as a preliminary bath about the fresh strip. Frequently it could be

TABLE VI.

Effect of a brief preliminary bath of fresh terrapin blood upon the latent period of a fresh strip of terrapin ventricle in 0.7 per cent NaCl.

Date of observation. 1903.	Latent period of control in 0.7 % NaCl.	Latent period of test strip.
Part 1.—Blood clotted on the strip.		
Feb. 11	minutes 26	minutes 16
" 21	30	15
" 28	35	10
Part 2.—Blood did not clot on the strip.		
Feb. 9	45	72
" 11	40	39
" 12	41	56

kept about the strip for three minutes, and then be withdrawn without clotting. In every case in which this was done, and the blood adhering to the strip was removed with care by thorough rinsing, no shortening of the latent period was observed. On the other hand, if the blood were allowed to clot about the strip before being removed, the latent period was shortened in the same way as when serum was used. The experiments with blood are recorded in Table VI. The average latent period for nine observations with serum, clotted plasma, and clotted blood was, for the test strips, twelve minutes, and for the controls, thirty-six minutes. These experiments indicate clearly that

the calcium in the normal uncoagulated blood is in a condition different from that in which it exists in the serum after coagulation. In the latter state it behaves, so far as the relationship that we are now studying is concerned, like the aqueous solution of a calcium salt, and it is possible, therefore, that the difference here pointed out between the calcium of the fresh plasma and of the serum may be connected with the degree of ionization that it undergoes in the two conditions.

Summary of the important conditions affecting the latent period.—

1. A mixture of 0.7 per cent sodium chloride with the proper proportion of calcium chloride, followed in a few minutes by 0.7 per cent sodium chloride alone, induces spontaneous beats in a freshly isolated ventricular strip more promptly than any other means now known; the average time is about ten minutes.

2. Spontaneous beats begin in a fresh strip in 0.7 per cent sodium chloride after a latent period four to five times as long as in Case 1.

3. Spontaneous beats begin in a fresh strip in sodium chloride diluted with isotonic sugar solution after a latent period three times as long as in Case 2.

4. Under certain conditions of the tissue, the latent period of fresh strips in 0.7 per cent sodium chloride solution is less than half the normal. Under these same conditions calcium, in slightly excessive doses, may exert an inhibitory rather than an accelerating influence.

5. The calcium-content of normal, uncoagulated plasma is in such a form that it does not exert the stimulating influence of inorganic calcium salts in solution upon freshly isolated ventricular strips, but during the process of clotting the calcium of the serum becomes efficient in this respect.

THE REACTIONS OF STRIPS IN 0.7 PER CENT SODIUM CHLORIDE SOLUTION.

The first effect of placing a freshly prepared ventricular strip in 0.7 per cent sodium chloride is a marked loss of tone. At the outset this is probably merely the normal relaxation from the condition of excessive tone into which the process of preparation has thrown the tissue. This large initial fall is succeeded by a gradual but continuous fall which continues till the death of the strip, if it be left in the sodium chloride solution that long.

Spontaneous beats begin in the strip after a latent period which has been sufficiently described in preceding paragraphs. The series in 0.7 per cent sodium chloride has a perfectly characteristic form, which has been fully described by Greene.¹ In brief it is as follows: The contractions may or may not show a distinct "Treppe," but after the maximum height is reached there is invariably a gradual continuous decline in the extent of contraction, terminating in a condition of practical standstill, which is known commonly as sodium chloride exhaustion. The duration of the series in 0.7 per cent sodium chloride shows wide variation. In the author's experiments it ranged from one-half hour to four hours; the average duration for sixty-eight observations was one hour, fifty minutes. In determining the length of these series, the strip was considered at rest when its actual shortening was less than 0.1 mm. Undoubtedly minute contractions continued for some time after that point was reached, which would make the average given above too small; for the purpose of this study, however, the figure given is sufficiently accurate. The extent of shortening of a strip in 0.7 per cent sodium chloride corresponds, at its maximum, about with that shown by fresh strips in air when stimulated electrically. It is by no means the utmost which the strip can be made to show under proper conditions.

As has been proved repeatedly, complete sodium chloride exhaustion does not imply that the tissue has suffered permanent injury, inasmuch as it may be revived by suitable means, and kept in vigorous activity for many hours. Continued exposure to the sodium chloride solution, after the exhaustion has become complete, does result, however, in the death of the tissue in a comparatively short time. Three distinct methods of restoring the activity of a strip which has come to rest in 0.7 per cent sodium chloride are known to the author.² These are: (1) the addition of a small proportion of calcium chloride to the solution in which exhaustion has taken place (the concentration of calcium chloride usually employed varies between 0.025 per cent and 0.05 per cent); (2) the transfer of the strip to an isotonic cane sugar, or similar "indifferent," solution; (3) removal of the strip to a moist air chamber.

The manner of recovery by each of these methods is quite characteristic of the method, and the variation undoubtedly depends upon the different changes which are brought about in the tissue as the

¹ GREENE: *Loc. cit.*, p. 91 *et seq.*

² HOWELL: *Loc. cit.*, pp. 185, 189; LINGLE: This journal, 1902, viii, p. 83.

result of the different environments, all leading to the same result, *i. e.*, renewed vigor of contraction. The effect of adding calcium chloride to the solution of 0.7 per cent sodium chloride, in which the strip has come to rest, is a very prompt and marked increase of vigor. The strip usually shows an increased beat in the very first contraction it makes after the addition of the calcium chloride, while within a half-dozen contractions the size of the beat will be considerable; sometimes nearly as great as at the beginning of the sodium chloride series. There is usually a prompt rise in tone as the result of the addition of calcium chloride; where the amount of the calcium salt is relatively large, this tonic shortening may be considerable. When a strip, exhausted in sodium chloride, is transferred to an isotonic sugar solution, the effect is more gradual, although it usually begins to develop within a short time after the application of the sugar solution. The increase in beat is slow but continuous, reaching its maximum about one half-hour after the first indication is given. Sometimes there is a slight rise of tone in the sugar solution, but not usually. The reviving effect of placing the exhausted strip in a moist chamber becomes apparent after a much longer interval than by either of the other methods described. In only one of the author's experiments could any change be detected in less than ten minutes, while in several instances an hour elapsed before the least increase in vigor could be seen. Once established, however, the increase in beat is continuous till the maximum is reached. This may be in from two and one-half to ten hours; the average of fifteen observations was about five hours. The ultimate vigor attained by strips, revived in moist chambers, usually equals the maximum exhibited by them in 0.7 per cent sodium chloride, and frequently exceeds that value.

The revival brought about by the addition of calcium chloride to the exhausting solution must be considered as due directly to the presence of calcium, since in no other respect has the solution been altered. The amount of calcium chloride which must be added to produce revival is too small to cause any measurable change in the osmotic relations of the solution, or in the proportion of chlorine ions in it. The revival which results from placing the strip in sugar solution may be explained as a direct effect of the sugar, or else as due to the diffusion of all salts, sodium chloride with the rest, out of the tissue. Sugar, as a non-electrolyte, is generally considered to be without specific action of the sort under discussion. If this assump-

tion be allowed, the revival in sugar is to be traced, perhaps, to the free diffusion of all salts which it permits. The revival in moist air would seem to be due to the direct application of the oxygen of the air to the tissue, or else to be related to the fact that this treatment brings to an end all possibility of either inward or outward diffusion. Lingle¹ suggests that the strip in sodium chloride exhaustion is in a state of mild asphyxia which is relieved by the transfer to air. Experiments will be cited presently, in another connection, in which strips beat forcibly and continuously for very many hours in other solutions whose volume was exactly the same as that of a 0.7 per cent sodium chloride solution which produced exhaustion in two hours. Moreover, the experiment of immersing the strip in a rapidly flowing stream of 0.7 per cent sodium chloride was carried out by the author, with no apparent change in the onset of exhaustion. These experiments make it very improbable that lack of oxygen has anything to do with sodium chloride arrest, or that supplying oxygen freely is the cause of revival in moist chambers. Lingle² lays great stress upon the remarkable revival which results from placing an exhausted strip in an atmosphere of pure oxygen. This revival differs, however, not at all in kind, but only in degree from that seen in ordinary air. That oxygen exerts an influence upon the metabolism which lies at the basis of all contractions, can be readily granted. That the mere presence of oxygen is sufficient of itself to cause contractions in the quiescent strip is yet to be proven. Lingle's attempt to prove it³ by adding hydrogen peroxide to the exhausting solution is insufficient, because the strip, under these conditions, becomes completely enclosed in a layer of bubbles; it is in effect removed from the solution, entirely so, so far as diffusion relations are concerned. The experiment becomes, then, merely another instance of revival by suspension in a moist chamber. In a later paragraph an attempt will be made to show that the revival which occurs in moist air can be satisfactorily explained upon the other alternative which was suggested above, *i. e.*, the elimination of all possibility of diffusion.

¹ LINGLE: *Loc. cit.*, p. 98.

² LINGLE: *Loc. cit.*, p. 83.

³ LINGLE: *Loc. cit.*, p. 81.

THE REACTIONS OF STRIPS IN MIXTURES OF 0.7 PER CENT SODIUM CHLORIDE WITH ISOTONIC SUGAR SOLUTION.

After the initial relaxation, which, as noted in a previous paragraph, is the first phenomenon exhibited by a freshly prepared strip of ventricle after suspension, the tone changes shown by strips in mixtures of sodium chloride and sugar solution depend upon the relative concentration of the two substances in solution. Where the proportion of sodium chloride is rather large the tone falls continuously, as it does in pure salt solution. With the sodium chloride quite dilute, the relaxation may come to an end soon after the strip resumes what may be called its normal length. The author has never observed any increase in tone, so long as some sodium chloride was in the solution, although it occurs constantly in pure sugar solution.

Sufficient mention has already been made of the fact that in the solutions under discussion the latent period is always much longer than in solutions of 0.7 per cent sodium chloride. The series of contractions given by strips in these mixed solutions of sugar and sodium chloride show some points of interest. In the first place, the extent of the initial beats of the series is always much greater than in any other environment with which the author has worked. Under favorable circumstances these initial contractions in dilute salt solution have been almost twice as high as those given by the same strip under electrical stimulation, when freshly isolated and hanging in the air. This great initial vigor is so marked that it cannot escape attention, and should be taken into account in considering the conditions of the heart's activity. The general form of curve given by strips in salt-sugar solutions is similar to that of strips in pure sodium chloride solution. The series, however, is always much longer; in the author's experiments the average duration of series in solutions of sugar mixed with salt was eight and one-half hours, compared with about two hours for the controls in 0.7 per cent sodium chloride. The longer duration is compensated largely by the slower rate of contraction. In one experiment the control in 0.7 per cent sodium chloride gave one thousand contractions in two hours, at the end of which time it was completely exhausted; the companion strip, in sodium chloride mixed with sugar solution, had given thirteen hundred contractions in eight hours when the drum stopped, so that the rest of the record was lost. At the end of the eight hours the strip was by

no means exhausted, although its contractions were quite small. The series usually do not show the regularity of sequence that is so characteristic of the beat in pure sodium chloride. In minor details they may exhibit various modifications. Exhaustion is sometimes accompanied by a gradual decline of the vigor of contraction to zero; in other cases the strip stops beating while the force of the individual beats is considerable. Recovery after a strip had come to rest in a sugar-salt solution was obtained by the author in three ways: (1) by transferring the strip to 0.7 per cent sodium chloride. In the two experiments in which recovery was obtained in this way it was prompt in showing itself, the first beat was more vigorous than any that succeeded it, though by no means so strong as those given by the same strip in the early part of the series in the mixed solution, and the length of the series in the pure salt solution was about the average for this solution. The form of curve was typical for 0.7 per cent sodium chloride, save for some irregularity of rate. (2) By transferring the strip to a moist chamber. The revival by this means was exactly similar, in the two cases in which it was tried, to that shown under the same environment by strips exhausted in 0.7 per cent sodium chloride. (3) By transferring the exhausted strip to a solution containing 0.7 per cent sodium chloride and 0.025 per cent calcium chloride. In two cases the revival by this means resembled that brought about by pure sodium chloride, in that the first contraction after recovery was greater than any succeeding ones, although in other respects there was no marked similarity. In two other cases there was a gradual increase of beat from zero to the maximum. In three of the four cases there was marked rise in tone.

THE REACTIONS OF STRIPS IN SOLUTIONS CONTAINING, IN ADDITION TO 0.7 PER CENT SODIUM CHLORIDE, FROM 0.025 PER CENT TO 0.06 PER CENT CALCIUM CHLORIDE.

Fresh strips placed in solutions containing sodium and calcium chlorides in the proportions given above, show no loss of tone other than the normal initial relaxation. In nearly every instance a rise in tone comes on after the strip has been in the solution for some time. If the proportion of calcium chloride is sufficiently great, this tonic contraction may become excessive, the condition being described by Howell¹ as calcium rigor. The series of spontaneous contractions

¹ HOWELL: This journal, 1902, vi, p. 187.

given by strips in these sodium-calcium solutions differ chiefly from those in pure sodium chloride solution in their longer duration. The range of length of series in the author's experiments was from four and one-half to forty and one-half hours; the average duration of twelve series was seventeen hours. When strips which have been exhausted in 0.7 per cent sodium chloride solution are revived by the addition of calcium chloride to the solution, the series which result are essentially similar to those given by fresh strips in solutions of sodium and calcium chlorides. The average duration of five such series was twenty hours. Strips which have been exhausted, first in 0.7 per cent sodium chloride, and then in isotonic cane sugar, and have then been placed in a sodium-calcium solution, show in this solution the same sort of long-continued series as in the same solution after other preliminary treatment, such as is described above. A noteworthy fact in regard to the effect of solutions of sodium and calcium chloride upon strips, is that when a strip has come to rest in such a mixed solution, it appears to be in a condition from which it cannot be recovered. Neither transfer to pure 0.7 per cent sodium chloride, nor to isotonic cane sugar, nor to moist air has any reviving effect, although, as will be shown, no other kind of exhaustion studied by the author failed to be overcome by one or the other of these methods.

THE REACTIONS OF STRIPS IN NORMAL RINGER'S SOLUTION.

The statement was made in a previous paragraph that fresh strips, placed in Ringer's solution, do not usually show spontaneous contractions. Occasionally, however, they may do so. The author observed this result three times in the course of his experiments. Each of these occurred during warm weather. The contractions were vigorous but infrequent; the average duration of the series was six hours. In this, as in every observation of series of contractions in Normal Ringer, the end of the series was marked by increasing length of interval between contractions, without any marked diminution in the extent of the individual beats. Cessation of activity seemed more like the result of an inhibition than of such exhaustion as occurs in 0.7 per cent sodium chloride.

When a strip, after exhaustion in sodium chloride solution, is placed in Ringer's fluid, the first effect corresponds exactly with that observed when revival is brought about by the addition of calcium

chloride to the exhausting solution; there is a prompt increase of extent of contraction. After a short time, however, the rhythm becomes irregular and the beats infrequent, as compared with series in solutions containing only sodium and calcium chlorides, without potassium chloride. A Ringer series after sodium chloride exhaustion is shorter than a corresponding series in a sodium-calcium solution. The average length of series in Ringer's fluid, after sodium chloride exhaustion, was, for five observations, twelve hours; against twenty hours for series in a sodium-calcium solution under corresponding conditions.

Transfer to Ringer's solution after successive exhaustion in 0.7 per cent sodium chloride and isotonic sugar solution, is followed by essentially the same activity as after sodium chloride alone. In the author's experiments the average length of the series after this double exhaustion was four hours more than when the transfer was made directly after sodium chloride exhaustion. Strips which have come to rest in Ringer's solution may be revived with the greatest promptness by transferring them to 0.7 per cent sodium chloride solution, or to a mixture of that solution with calcium chloride in physiological concentration. These results were obtained by the author with strips which had been active in Ringer's fluid for forty hours. The extent of contraction after revival is the same as that shown in the final beats executed by the strip in the Ringer, and these latter may equal the most vigorous beats given in any part of the series, or they may be somewhat less powerful. These facts seem to indicate strongly that cessation of activity in Ringer's solution is a potassium effect.

THE REACTIONS OF STRIPS IN A MOIST CHAMBER.

Freshly isolated strips, as has been shown, do not usually exhibit spontaneous activity if suspended directly in moist air; if, however, the strip be subjected to the proper treatment before being placed in the moist chamber, very long and excellent series of contractions may be obtained. If a strip be suspended in 0.7 per cent sodium chloride, or in a sodium-calcium solution, until it executes a single contraction, and be then quickly transferred to a moist chamber, a series of beats is obtained similar to those given by strips in sodium chloride solution in that the force of contraction declines continuously from the beginning, but differing from the series in such solutions in being considerably longer, and also by the fact that the contractions become

more and more infrequent toward the end of the series. The greatest length of series observed by the author under these conditions was fourteen and a half hours; the average for seventeen observations was four hours. Strips which have been placed, thus, in moist air at the very outset of their activity, and have proceeded to exhaustion in the moist chamber, may be recovered well by being returned to the solution in which activity was first induced. An interesting point about the recovery that is brought about by the use of 0.7 per cent sodium chloride is that in several cases, under the conditions now being considered, it began with feeble contractions which improved gradually till the maximum was attained. In every other instance in which the author observed either the initiation or the strengthening of beats by means of sodium chloride, the very first contraction after the application of the salt solution was either maximal or slightly submaximal. Recovery was also obtained by placing the strip in a solution containing 0.05 per cent calcium chloride in 0.7 per cent sodium chloride, and in a single instance by the momentary application of a solution of 0.05 per cent calcium chloride in sugar solution, isotonic with 0.7 per cent sodium chloride.

Reference has already been made to the excellent series of contractions that can be obtained by removing strips which have been completely exhausted in 0.7 per cent sodium chloride to moist air. These series ranged, in the author's experiments, from seven to twenty-seven hours in length; the average for thirteen observations was sixteen hours. The maximal force of contraction, which was reached four to eight hours after the transfer to the moist chamber, was frequently as great as that shown by the strip at the beginning of the sodium chloride series. For the first few hours after being placed in the moist chamber the rate of beat was rapid and regular. In the later stages of the series, however, it became slow and quite irregular. The appearance of the tracing taken during the latter part of a moist chamber series resembles very closely that given by a strip beating in Ringer's solution. The end of the moist chamber series was always marked by the long intervals between the final contractions. Frequently there was also a gradual decline in the force of the contractions during the later stages of the series, but in a number of instances the only indication of exhaustion was the growing infrequency of the contractions. Strips which have come to rest after hours of activity in moist air may be revived in various ways. One of these is by returning the strip to 0.7 per cent sodium chloride.

In five experiments, in which the duration of the series in the moist chamber varied between sixteen and twenty-seven hours, recovery was obtained in this way. In two cases the strip had come to rest after a period of lessening frequency without any diminution in the force of the beats. In both these cases the vigor after revival was as great as at the end of the moist chamber series. In three cases the force of contraction had declined gradually to zero before the transfer to 0.7 per cent sodium chloride. In these cases the maximum vigor after revival was markedly less than the greatest previously shown by the strip. In every instance the series in the salt solution was typical of the solution; in other words, there was a continuous decline in the extent of the contractions. The length of the series varied from twelve to fifty-five minutes; the average for the five observations was twenty-eight minutes. Another method of reviving a strip after exhaustion in a moist chamber, is by treating it with mixtures of calcium and sodium chlorides in solution, using the same proportion of calcium chloride as in the other experiments reported. The recovery by this means is characterized by the vigor of the resulting contractions, which is much greater than that shown by strips recovered by sodium chloride alone, by the long duration of the series after recovery, by the rise in tone which usually occurs, and by the fact that recovery ensues very promptly after the application of the calcium chloride to the strip.

A fact of interest in connection with the activity of ventricular tissue in moist air, is the remarkable stimulating influence which is exerted by solutions containing calcium, when such solutions are allowed to drip over the surface of the tissue.¹ The author used for this purpose solutions of calcium chloride in sodium chloride solution; of calcium chloride in isotonic sugar solution; and specimens of ordinary well-water, which showed a strong calcium test with ammonium oxalate. The method of application was as follows: a few drops were forced from a pipette upon the silk thread connecting the muscle with the recording lever, whence they ran down over the surface of the tissue. The reviving effect was observed repeatedly, and at various stages of the moist chamber series. If the strip was in a state of exhaustion, either after sodium chloride or after a long period of activity in a moist chamber, the effect corresponded with that shown when a bath containing calcium is applied to the strip;

¹ GREENE: *Loc. cit.*, p. 107.

the extent of contraction was promptly and markedly increased. If the strip was beating with vigor, but at long intervals, the effect of moistening it with the calcium-containing solution was to improve the rate and rhythm. That this is a calcium effect solely is indicated by the fact that the revival caused by calcium chloride in sugar solution, or by the calcium-containing water, could not be distinguished from that which resulted from the use of mixtures of calcium and sodium chlorides; moreover, similar moistening with 0.7 per cent sodium chloride alone was absolutely without effect, although it was tried repeatedly in circumstances in which moistening with a calcium-containing solution, or even *immersion* in a solution of sodium chloride, would almost certainly have resulted in recovery. Although the application of calcium is transient, the effect is usually prolonged. In a number of instances in the author's experience a strip thus revived showed the beneficial effect of the treatment for five or six hours after its application. It should be borne in mind, in this connection, that while these calcium effects may result from a very momentary action on the part of the calcium-containing solution, initiation of beats or revival of vigor brought about by sodium chloride alone, invariably requires that the strip be immersed in the sodium chloride solution.

A number of observations were made on the effect of placing a fresh strip in a moist chamber after a brief immersion in 0.7 per cent sodium chloride, or the same solution containing also 0.05 per cent calcium chloride. The latter solution was left about the strip for five minutes in every case; the former was left for variable times up to fifteen minutes, but was always removed before the onset of activity. Strips in moist air, after brief immersion in a calcium-containing solution, showed spontaneous activity in six cases out of eight that were studied. The contractions were infrequent in every case, and came at irregular intervals, although the individual contractions were very vigorous. The average duration of the series was about one and one-half hours. No decline in vigor occurred at its close. After the strip had stopped beating, moistening it with a calcium-containing solution usually called forth four or five vigorous beats close together, but had no further apparent effect. Transfer to a 0.7 per cent sodium chloride solution promptly resulted in a good sodium chloride series. In only two cases was the author able to elicit spontaneous contractions from strips which had been removed from 0.7 per cent sodium chloride to moist air before the

.

onset of activity. In each of these cases the total number of contractions was very small, and they appeared at long intervals.

THE REACTIONS OF STRIPS IN CANE SUGAR SOLUTION ISOTONIC
WITH 0.7 PER CENT SODIUM CHLORIDE SOLUTION.

Fresh strips placed in sugar solution show only tone changes. There is at the outset the usual relaxation, which is followed by a gradual and continuous increase in tone; this tonic shortening may amount ultimately to a very considerable fraction of the original length of the strip. When a strip in this tonic contraction is removed to 0.7 per cent sodium chloride, a prompt fall in tone occurs, and unless the time of immersion in sugar has been excessive, spontaneous contractions will begin, sooner or later. In none of the author's experiments was the onset of activity after the application of 0.7 per cent sodium chloride markedly prompt. If a fresh strip be placed in 0.7 per cent sodium chloride till beats begin, and be then transferred to sugar solution, activity ceases immediately, and the same rise in tone occurs as when a perfectly fresh strip is placed in sugar solution. It will be remembered that strips removed to moist air, under the same conditions, give excellent series of contractions. Howell¹ has described the effect of sugar solution upon strips which have been exhausted in sodium chloride. Revival of vigor as the result of transfer to a sugar solution is usual, but not invariable. When revival occurs it is gradual and continuous until a maximum is reached which is somewhat smaller than the maximum in sodium chloride. Usually no rise in tone occurs. The series in sugar, which lasts an hour on the average, terminates ordinarily without diminution of vigor, but the last few beats are at longer intervals than the others. In a few of the author's experiments the vigor declined gradually to zero. When a strip that has come to rest after a series in sugar solution, is placed again in 0.7 per cent sodium chloride, it usually gives a series of contractions like that ordinarily seen in sodium chloride, save for the exceedingly rapid decline in vigor which occurs. In the author's experiments, the longest series given by a strip in sodium chloride after exhaustion in sugar was twenty minutes. If the strip be returned to the sugar solution after this second sodium chloride exhaustion, a second good recovery and series may ensue. This may, in turn, be followed by a short series

¹ HOWELL: *Loc. cit.*, p. 185.

in sodium chloride. The author has not succeeded in carrying the alternation of sodium chloride and sugar farther than this with good results. The effect of solutions containing both sodium and calcium chlorides, or of Ringer's solution, upon strips exhausted in sugar, is, as Howell¹ has pointed out, extremely beneficial. Excellent series of long duration may be obtained by the use of these solutions; in the author's experiments the duration of series under these conditions ranged from six to forty hours; the average was about eighteen hours. If a strip which has been exhausted in sugar be transferred to a moist chamber, it frequently revives, and gives a long and excellent series of contractions. These series showed, in the author's experiments, about the same range of duration, and the same average duration as was noted above for strips in solutions containing both sodium and calcium chlorides. This result was obtained repeatedly, and is interesting inasmuch as a strip after exhaustion in sugar has been assumed to be wanting in all diffusible ions. Moistening the strip with a sugar solution containing calcium, at the moment of placing it in the moist chamber, was without immediate effect in two instances, save for a slight rise in tone. The beats, which began after a short latent period, were vigorous, but not demonstrably more so than in cases where no calcium had been applied. In neither of these instances was the length of the series widely different from the average. In one case, however, a strip that had been exhausted successively in sodium chloride and sugar, and had then been beating feebly in a moist chamber for two and one-half hours, was moistened with a sodium-calcium solution, containing 0.05 per cent calcium chloride. The force of contraction increased promptly and markedly as a result of this application, and the strip remained active for twenty-five hours after the application of the reviving solution.

After a strip has been exhausted successively in 0.7 per cent sodium chloride, isotonic sugar solution, and moist air, and has given a good series of contractions in each, it may then be revived by being placed in a solution of sodium and calcium chlorides, and may give an excellent and vigorous series in this solution. The figures for two such experiments are as follows:

Experiment of Dec. 17, 1903. — A strip was exhausted in 0.7 per cent sodium chloride by 1.5 hours' immersion. It was then placed in isotonic sugar solution, in which it made a good recovery and gave a typical series of

¹ HOWELL: *Loc. cit.*, p. 190.

the usual length. At the end of 1.5 hours after being put into the sugar solution, it had become perfectly quiescent. It was then transferred to a moist chamber, where it recovered a second time, and remained active for more than seventeen hours. Eighteen hours after being placed in the moist chamber the strip had again come to rest. It was then put into a solution of sodium and calcium chlorides. Vigorous beats commenced at once, and the strip remained active for twelve hours.

Experiment of Dec. 21, 1903.—A strip was exhausted successively in 0.7 per cent sodium chloride, isotonic sugar solution, and moist air, giving a typical series of contractions in each medium. It was in the salt solution two hours, in the sugar solution one hour, and in the moist chamber twenty-one hours. At the end of this treatment it was immersed in a sodium calcium solution, containing 0.05 per cent calcium chloride and 0.7 per cent sodium chloride, and gave a good series of beats lasting fifteen hours.

SUMMARY.

The following reactions seem to the author to be normal for ventricular tissue of the turtle, and in his opinion must all be taken into account in any attempt to formulate a satisfactory theory of the rhythmicity of such tissue.

1. Ventricular tissue of the turtle's heart is not spontaneously rhythmical under the conditions of normal life. This is shown by the fact that it ceases to beat when completely isolated.

2. It contains within itself all the conditions essential for rhythmic activity, provided they can be brought into play.

3. By subjecting the tissue to certain artificial conditions it can be made spontaneously rhythmical; the most important of these artificial conditions are stated in the succeeding sections.

4. A sodium salt in some proportion must be present in any solution which is to be used as a bath about a ventricular strip for the purpose of initiating spontaneous contractions in it.

5. A brief preliminary bath of a solution containing calcium in physiological proportion, followed by immersion in 0.7 per cent sodium chloride, induces spontaneous beats in a fresh strip more promptly than any other solution now known.

6. Fresh strips will begin to beat spontaneously if treated only with 0.7 per cent sodium chloride, but much more time elapses before the onset of activity in this case than in the former one.

7. Spontaneous contractions may occur in solutions containing a much smaller amount of sodium chloride than that usually consid-

ered as physiological, if the solutions are kept isotonic by the addition of some "indifferent" substance, such as cane sugar, but the latent period in this case is much prolonged over that which occurs in the other cases mentioned.

8. Long continuance in the abnormal conditions incident to complete isolation from the body, modifies the tissue so that its general sensitiveness to the influence of salts is markedly increased.

9. The series of contractions in 0.7 per cent sodium chloride is characterized by continuous diminution of vigor, resulting finally in standstill, the so-called "sodium chloride exhaustion."

10. Recovery from sodium chloride exhaustion may be secured by adding calcium chloride to the solution, by permitting free diffusion of the electrolytes present in the heart-muscle, or by preventing diffusion completely. The recovery by the second method is comparatively short-lived, while that brought about by the first and third methods is very long continued.

11. Spontaneous activity in isotonic solutions containing small proportions of sodium chloride is characterized by the extreme vigor of the initial contractions. The rate is much slower than in 0.7 per cent sodium chloride, but this is fully compensated by the much greater length of the series of beats.

12. Recovery from the exhaustion which occurs in dilute sodium chloride solutions may be obtained by the application of 0.7 per cent sodium chloride, by the application of mixed solutions containing both sodium and calcium chlorides, or by the complete stoppage of diffusion which results when the tissue is placed in a moist chamber.

13. Spontaneous activity in solutions containing both sodium and calcium chlorides is characterized by the very great length of the series of beats, and also by the fact that exhaustion in such solutions is not overcome by the methods applicable to other forms of exhaustion.

14. Spontaneous activity in Ringer's solution is characterized by the great length of series it induces, and by the irregular rhythm exhibited by strips immersed in it. Cessation of activity in this solution appears more like inhibition than like true exhaustion.

15. Recovery from standstill in Ringer's solution may be obtained by the application of pure sodium chloride, or of mixtures of sodium and calcium chlorides. The series of beats after recovery with the first-named solution shows the usual characteristic of a more or less

rapid decline to zero, while that with the second solution is very long and well sustained.

16. Spontaneous activity in moist air occurs only after it has been induced by treatment with one of the effective solutions described above. The nature of the series of contractions given in the moist chamber depends upon the previous treatment which the tissue has had. The most favorable preliminary treatment appears to be exhaustion in 0.7 per cent sodium chloride, or successive exhaustion in this medium and in sugar solution isotonic with it. The momentary application of calcium to a strip in moist air affects favorably both the vigor of contraction and the rhythm of the series. The later stages of a moist chamber series are characterized by irregularity of rhythm like that shown by strips in Ringer's solution. If the strip is removed to moist air at the very beginning of spontaneous activity, the resulting series is characterized by gradually declining vigor from the outset, as well as by the diminishing frequency of contraction which is usual in moist air. The series under such conditions is much shorter than when the moist chamber is applied after exhaustion in sodium chloride.

17. Recovery from moist chamber exhaustion may be obtained in precisely the same ways as from standstill in Ringer's solution. In this case, as in that, the recovery which results from the application of a mixed solution of sodium and calcium salts is much better than that which follows the use of sodium chloride alone.

18. Spontaneous activity in sugar solution, isotonic with 0.7 per cent sodium chloride, appears to occur only after previous exhaustion in sodium chloride. It is characterized by the short duration of the series, and by the fact that standstill frequently comes on with the extent of contraction still high.

19. Recovery from standstill in sugar solution may be obtained by the application of isotonic sodium chloride, in which case the recovery is very short-lived, or by the application of a sodium-calcium solution, or by the stoppage of diffusion which results from the removal of the strip to moist air; the recovery in the two latter cases is excellent and long-continued.

THEORETICAL DISCUSSION.

Study of the results described above leads to the conclusion that the simple theories thus far proposed in explanation of the reactions of ventricular tissue to salts, in which all the effects observed are

attributed to mere diffusion inward or outward of one salt or another, are insufficient to account for all the phenomena, and that the ultimate explanation of these effects will prove to be more complicated. Two sets of phenomena reported in this paper seem to be peculiarly instructive when studied together. The first of these has to do with the effect of calcium on the latent period, and the second with its effect on recovery from sodium chloride exhaustion. In the first case it is seen that spontaneous contractions appear promptly in a fresh strip treated with calcium and then placed in sodium chloride, while if the calcium treatment be omitted, and the fresh strip be placed directly in the saline solution, contractions appear only after a considerable lapse of time. The second case is analogous; a strip which has lost its irritability as the result of prolonged immersion in salt solution is recovered with great promptness by the application of calcium, and exhibits an equally good recovery as the result of removal from the sodium chloride solution to moist air, but only after a long interval. These facts may be taken to indicate that the result of immersion in sodium chloride, in the first instance, and of suspension in moist air, in the second, is to bring about gradually, by processes within the tissue, conditions equivalent to those obtained immediately by the application of calcium directly to it. The tentative hypothesis which the author offers in explanation of the observations of himself and others, along the lines under consideration, is based upon the idea expressed above. The first assumption suggested would be that the liberation of energy in ventricular tissue is dependent upon the presence in it of calcium in diffusible form, but that the normal calcium-content of the tissue is for the most part in some indiffusible form. According to this assumption, one effect of stimulation upon the tissue would be to convert the calcium from its indiffusible inert form to a diffusible active form. When a strip of ventricle is isolated from its normal connections, it may be supposed that the calcium-content passes over gradually from the inert to the active form, perhaps through the action of sodium salts upon the tissue, and that activity begins when sufficient diffusible calcium has accumulated to produce its effect. Although the assumed active calcium has been designated as diffusible, satisfactory explanation of some of the observed phenomena requires the assumption that the actual diffusion of calcium is comparatively insignificant when the tissue is at rest, but marked when it is in action.

The observations recorded in the author's summary must be

examined in their relation to this hypothesis, in order to see how far it is in accordance with the facts known at this time.

The latent period.—The effect of the external application of a calcium salt in shortening the latent period agrees with the idea that the presence of calcium ions is essential to activity, since this treatment provides them at once, and is followed by prompt activity. The idea has been advanced that the inert calcium-content of the tissue is rendered active suddenly as the result of stimulation. It may be supposed that sodium ions applied about the strip bring about a similar transformation gradually. From this point of view the latent period in a sodium-containing solution would be looked upon as an expression of the time required for sufficient transformation of calcium to take place to induce contractions. The fact that dilution of the sodium chloride bath with sugar solution prolongs the latent period can be explained by supposing that the rate of conversion of the calcium from its inert to its active form is dependent on the amount of sodium in the external environment.

Sodium chloride exhaustion, and recovery from it.—Upon the assumption that diffusion of the active calcium takes place with great readiness while the tissue is contracting, the decline of vigor in sodium chloride solution follows as a natural consequence. This solution offers no opposition to free calcium diffusion whenever the condition of the tissue permits it. It may be supposed that when the tissue is active diffusion of calcium goes on more rapidly than does the process of transformation into the active form. The result would be a gradual diminution in the quantity of effective calcium, as compared with the amount of sodium present, and an accompanying decrease of vigor. Addition of calcium to the solution in which exhaustion has taken place should have a prompt and favorable effect upon the vigor of contraction, because it acts to restore at once the balance between the sodium and calcium, upon which the execution of powerful contractions seems to depend. The beneficial effect of the presence of calcium in the bathing solution would be expected to be, as it is, long continued, since in this way the favorable proportion between the sodium and calcium is maintained. When an exhausted strip is transferred to a moist chamber, outward diffusion of calcium comes to an end, but the transformation into the diffusible form may be supposed to continue. The result will be a gradual accumulation of active calcium which will be accompanied by rhythmical contractions of increasing force. When a strip, after exhaustion in sodium

chloride, is placed in sugar solution, outward diffusion of sodium is possible, as well as of the other electrolytes. If it be supposed that sodium is highly diffusible, more so than the active form of calcium, the result of the transfer to sugar would be a *relative* increase in the amount of active calcium, as compared with the sum total of the other electrolytes of the tissue, and this relative increase in the amount of effective calcium could be looked upon as the cause of the increased vigor of beat.

Activity in sodium chloride diluted with isotonic sugar solution.—The characteristic features of activity in dilute sodium chloride solutions, namely, great vigor and slow rate, may probably be interrelated, as Lingle¹ has suggested. The cause of the slow rate is not improbably the same as of the long latent period, *i. e.*, the small proportion of sodium in the bathing solution, and presumably, as the result of diffusion, in the tissue also. If the possible output of energy of the strip is not affected by the diminution in the amount of sodium in it, the slower rate would naturally be compensated by greater force of contraction. It should be borne in mind in considering the effects of solutions containing sugar, that the possibility of direct influence on the part of this substance is by no means excluded, and while it is desirable, from theoretical considerations, to consider sugar solutions indifferent, the experimental results of Howell² and others indicate that they may exert a definite and specific influence upon tissues immersed in them. The most interesting of the methods for reviving strips which have come to rest in dilute sodium chloride, is the application of 0.7 per cent sodium chloride. The revival under this treatment is ordinarily very prompt in showing itself, indicating that whatever the direct influence of the sugar may be, it is quickly overcome. On the assumption that sodium influences the conversion of calcium to its effective form, the beneficial effect of the application of the strong solution of salt would be due to the fact that it brings this transformation about to an increased degree. At the same time the normal proportion of sodium would be restored in the tissue, and complete outward diffusion of the sugar would occur.

Activity in mixed solutions of sodium and calcium chlorides.—Howell³ has described and discussed the results of immersing heart strips for a long time in sodium-calcium solutions in which the pro-

¹ LINGLE: This journal, 1900, iv, p. 275.

² HOWELL: This journal, 1901, vi, p. 188.

³ HOWELL: This journal, 1901, vi, p. 187.

portion of calcium is somewhat greater than the physiological. He points out that the ultimate effect of this large dose of calcium is to throw the tissue into a contracted condition, which he designates as calcium rigor. He points out that after a strip has gone into this condition it cannot again be roused into action. In the present paper it was shown that in cases in which the proportion of calcium is not great enough to bring about calcium rigor, the cessation of activity is marked by the same characteristic of resistance to ordinary modes of recovery. This is what is to be expected on any theory which assumes that interaction between sodium and calcium is essential to the activity of this kind of tissue. If the proportions of sodium and calcium in the bathing solution are correct, activity might continue until the available supply of energy-liberating material is exhausted, and after that point is reached recovery can only be obtained, if at all, by renewing the supply of such material.

Activity in Ringer's solution.—This solution differs from the one just under consideration only in that it contains a small proportion of potassium chloride. The relative infrequency of contraction and irregularity of rhythm which characterize activity in this medium, can be most simply explained as Howell¹ has suggested, as due to the restraining and inhibiting influence of the potassium ions. The fact that cessation of activity is not accompanied by diminishing vigor, but only by lessened frequency of contraction, indicates strongly the influence of an inhibiting agent. That this agent is the potassium is indicated with equal clearness by the fact that the only treatment necessary for complete renewal of activity is the change from Ringer's solution to a precisely equivalent one in which no potassium salt is present.

Activity in moist chambers.—The fact that the freshly isolated ventricle ordinarily remains at rest in moist air can be explained as due to the inhibiting action of the potassium ions present in the tissue, or, according to the suggestion made in this paper, on the supposition that the calcium does not become effective except under the influence of sodium. It seems to me not unlikely that both these factors may be operative. All the methods by which spontaneous contractions can be initiated are explicable upon this assumption of a twofold cause of standstill, and it accords with the facts which are known as to the effects of variations of environment upon the spon-

¹ HOWELL: *Loc. cit.*, p. 201.

taneous activity of the strip, after it has been initiated. Two facts, first, that the later stages of series of contractions in moist chambers normally exhibit increasing irregularity of rhythm and diminishing rate, presenting, thus, more and more, the characteristic features of series in potassium-containing solutions, and, second, that cessation of activity under these conditions resembles inhibition, rather than exhaustion, seem to indicate that there may be a gradual accumulation of effective potassium ions, analogous to that which has been suggested for calcium. The methods for reviving strips which have come to rest after activity in moist air, may be interpreted as favoring such a view. As was noted in a previous paragraph, the modes of causing recovery after exhaustion in moist air are the same as those which are effective in overcoming Ringer standstill, namely, methods which provide for the removal of the excess of potassium by diffusion. A comparison of all the methods of treatment which result in a renewal of activity after standstill in moist air, shows that the failure of the strip to beat can hardly be due to lack of sodium or of calcium, or to excess of either of these substances, inasmuch as revival can be obtained by the application of mixtures of sodium and calcium, or by either one without the other. The most direct conclusion, in view of all these facts, is that excess of potassium is the cause of the cessation of activity in moist air. This conclusion involves the assumption of an accumulation of potassium, which can be most readily explained, it seems to me, by supposing the potassium to be present in the tissue in an ineffective form, analogous, perhaps, to that in which the calcium occurs normally, and that it undergoes a gradual transformation, by virtue of which it becomes effective.

Activity in sugar solution.— Spontaneous contractions seem to occur normally in solutions of sugar only after complete exhaustion in sodium chloride solution. Inasmuch as a bath of sugar solution allows, presumably, diffusion of all electrolytes from the tissue, and it is well established that the presence of electrolytes is one of the essentials of activity in heart-tissues, Howell's¹ assumption of a differential diffusion seems best adapted to explain the revival which occurs under these conditions. Since immersion in sugar solution does not cause beats under other circumstances, it must be supposed, granting that differential diffusion is the correct explanation in this case of the activity after sodium chloride exhaustion, that the condi-

¹ HOWELL: *Loc. cit.*, p. 198.

tion of the tissue, as regards its content in electrolytes, is not such that the differential diffusion in sugar will cause or revive contractions, save under this special circumstance of previous exhaustion in sodium chloride. According to the hypothesis in whose light the heart reactions are being considered in this paper, the end of the series in sodium chloride would be a favorable time for such a differential diffusion to be effective; the transformation of calcium to its active form is well established, the sodium content of the tissue is at a high level, and the previous bath of sodium chloride has permitted the outward diffusion of potassium. In fresh strips, or those that have been but a short time in sodium chloride solution, these favorable conditions do not occur. The cessation of activity in sugar after the short series of beats which occurs in this medium may be due to the disturbance of that relationship of the electrolytes which results in activity, through their continued outward diffusion, or it may be due to a direct effect of the sugar. The methods of restoring a strip to action after it has come to rest in sugar, particularly the transfer to moist air, which induces a most excellent recovery, seem to indicate that the standstill in sugar solution is due to disturbance of the electrolytic relationships, rather than to an injurious effect of the sugar.

Seasonal variations in the reactions of heart tissue.—The marked shortening of the latent period in sodium chloride exhibited by some hearts in the summer time, seems to me to be of sufficient interest to merit a brief discussion. The hypothesis under consideration supposes that irritability in heart-tissue depends upon the amount of transformation of its calcium-content from an ineffective to an effective form. It can be conceived that the ease with which this transformation may be induced differs with variations in external conditions, particularly, perhaps, with seasonal or temperature changes. If we suppose the inert calcium-content of the summer heart to be much less stable than that of the heart in cold weather, the gentle accelerating action of sodium in solution would bring about the transformation of the calcium in less time, with the resulting effect of shortening the latent period. It is difficult to imagine why increased instability of the normal calcium-content of the tissue should always be accompanied, as it seems to be, by an inhibitory influence on the part of large doses of calcium in the surrounding solution; but a more intimate knowledge of the relations of the various salts to the heart's activity will doubtless make this point clear.

BIOGRAPHICAL SKETCH.

Ernest Gale Martin was born in Minneapolis, Minnesota, November 16th, 1876. His father is a clergyman. He received his primary education in the public schools of the State of Minnesota, and in September, 1893, entered the academic department of Hamline University, St. Paul, Minnesota. He was graduated from that institution with the degree of Bachelor of Philosophy in June, 1897. From January, 1894, to June, 1895, he served as assistant in the biological laboratory of Hamline University. For the four years succeeding his graduation he taught elementary sciences in the high schools of Elk River and Austin, Minnesota. During the summers of 1897 and 1898 he was Demonstrator in Chemistry at the Chautauqua, New York, Summer College. He entered Johns Hopkins University as a graduate student in October, 1901, pursuing studies in Physiology, Zoology, and Physical Chemistry. He held the position of Fellow in Physiology during the year 1902-03, and of Assistant in Physiology during the year 1903-04.

Johns Hopkins University,
Baltimore, Maryland, April, 1904.



